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GROWTH OF L CELLS IN A CHEMICALLY DEFINED MEDIUM IN A CONTROLLED ENVIRONMENT CULTURE SYSTEM

Gordon W. Taylor
John P. Kondig
Stanley C. Nagle, Jr.
Kiyoshi Higuchi

MARCH 1970

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1B562602AD01 March 1970

GROWTH OF L CELLS IN A CHEMICALLY DEFINED MEDIUM IN A CONTROLLED ENVIRONMENT CULTURE SYSTEM*

ABSTRACT

Equipment has been developed to permit monitoring and automated control of environmental variables such as pH, temperature, pO_2, pCO_2, and redox potential in order to study their effects on the growth and metabolism of cultured mammalian cells. A battery of six water-jacketed 500-ml Bellco spinner flasks was instrumented to provide (by electrode probes) information on pH, pO_2, and redox potential of each culture during growth. Stepping switches and motorized valves coupled to the sensing probes permitted control of the environment. Studies with automated control of pO_2 levels in L cell cultures showed that dissolved O_2 tensions of about 9% were optimal for cell growth. At pO_2 values of 5 and 20%, peak cell yields as well as growth rates were reduced by approximately 20%. Peak yields of L cell cultures exceeded 5×10^6 cells per ml when grown for 4 days without medium renewal from inocula of $1.0 \pm 0.05 \times 10^6$ cells per ml in a defined medium sparged with 5% CO_2 and adequate O_2 to maintain 9% dissolved O_2 tension. The redox potentials of L cell cultures reflected the pO_2 levels in the medium and ranged from -25 to +150 mv (calomel reference) for O_2 values ranging from 2 to 20% dissolved oxygen tension.

Workers in cell culture research have long recognized that factors such as temperature, pH, O_2 partial pressure, CO_2 partial pressure, and redox potential have significant effects on cell physiology. Cooper,[1] Paul,[2] Daniels,[3] McLimans,[4] Kilburn and Webb,[5] and Moore,[6] as well as many others, have employed various procedures to study the effects of one or more of these factors on the growth and metabolism of cultured mammalian cells. We believe, however, that much more remains to be done in order to understand fully the effects of these variables on cellular physiology.

Equipment developed here permits studies of effects of varied environmental conditions on mammalian cells grown in a chemically defined medium. Results obtained in this system concerning the effects of varied oxygen tension on cell growth and related data on culture pH and redox potential are included here.

The cell employed in these studies was a substrain of the mouse fibroblast cell strain L described and designated L-DR by Daniels. The culture medium was the chemically defined medium of Nagle et al.,[7] modified as indicated by recent studies in our laboratory (Table 1).

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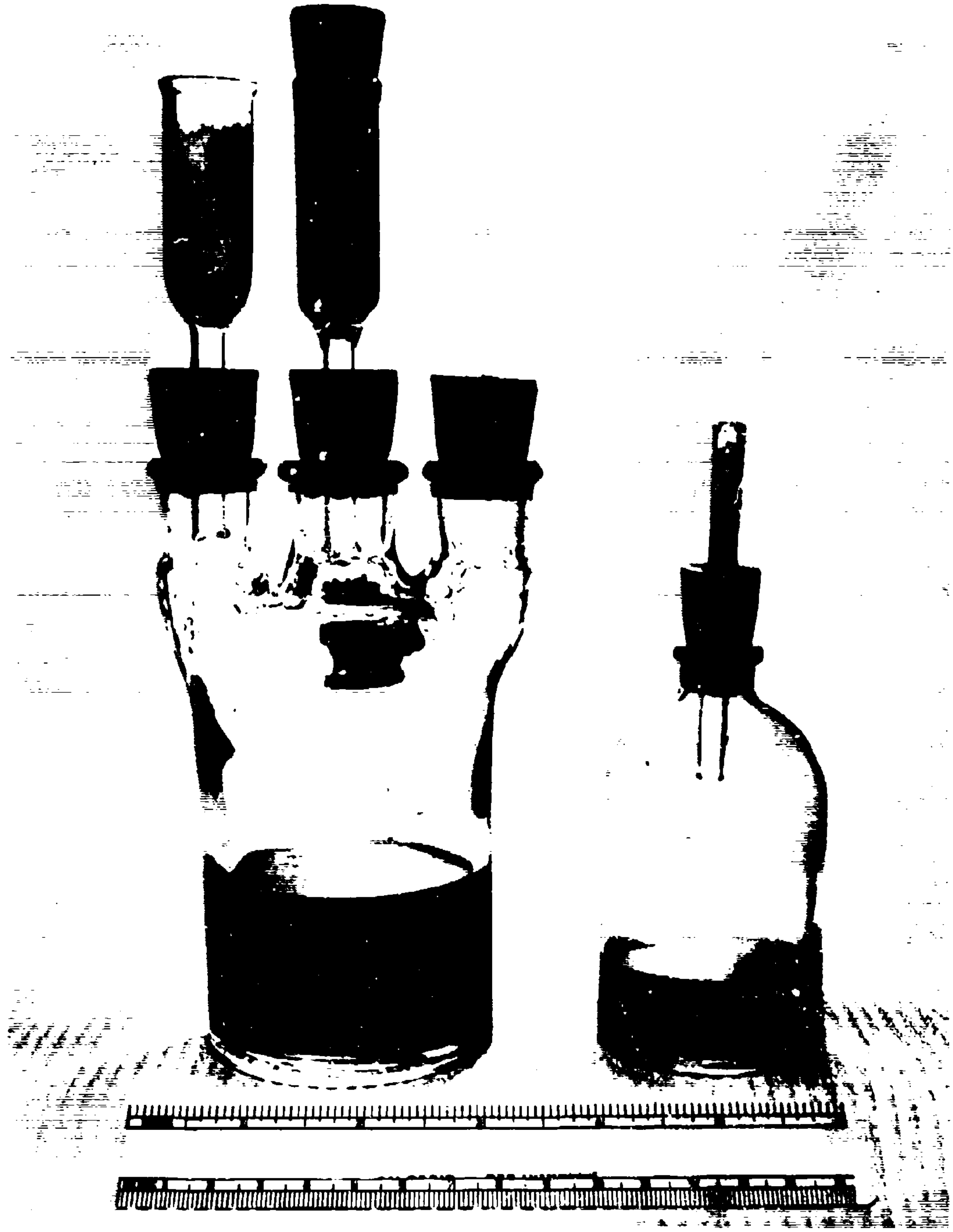
TABLE 1. A MODIFIED, CHEMICALLY DEFINED MEDIUM FOR THE GROWTH OF L CELLS IN SUSPENSION

Component	Per Liter
USA #1 (2X basal), Nagle[7]	500 ml
Vitamin mixture (100X)	10 ml
Choline chloride	48.0 mg
L-Proline	115.0 mg
L-Serine	105.0 mg
L-Asparagine	150.0 mg
$NaH_2PO_4 \cdot H_2O$	176.0 mg
Iron (as ferric ammonium citrate$\cdot 3H_2O$)	3.14 mg
Glucose	2000 mg
Methylcellulose (15 centipoise)	1000 mg
Kanamycin/Penicillin	50 mg/100,000 units
Insulin (zinc insulin)[a]	50 units
5% Sodium bicarbonate to pH 7.0	

a. May be replaced with 10^{-6} M $ZnSO_4 \cdot 7H_2O$.

Stock cultures of L cells were maintained at cell populations ranging from 1 x 10^6 to 10 x 10^6 cells per ml in 25 ml of medium in 100-ml serum bottles on the gyrotory shaker at 35 C with daily medium replacement. Inocula for our larger culture vessels were grown in shaken 250-ml Woulff bottles containing 100 ml of culture medium (Fig. 1). These vessels required venting when populations exceeded 10^6 cells per ml because of the need for more adequate gas exchange. Excellent pH control was thereby obtained because the escape of metabolic CO_2 prevented excessive drop in pH of the cultures.

The experimental cultures were inoculated with 1.00±0.05 x 10^6 cells per ml and were grown in a battery of six water-jacketed, 500-ml Bellco spinner flasks, arranged on a table with magnetic stirrer drives. The temperature in the culture vessels was maintained at 35 C with a Haake constant-temperature water circulator. Each spinner flask was constructed with four ports (Fig. 2). A Yellow Springs oxygen probe was inserted in one port, a platinum electrode and a combination pH probe were inserted in the second and third ports. All probes were chemically sterilized in acid-alcohol for 10 minutes, rinsed in sterile distilled water, and aseptically inserted into the flask. The remaining port was used for sampling and gas sparging lines, and to vent exhaust gases.



F GURE 1. L Cell Cultures in Vented Wouiff (left) a: d Serum (right) Bottles.

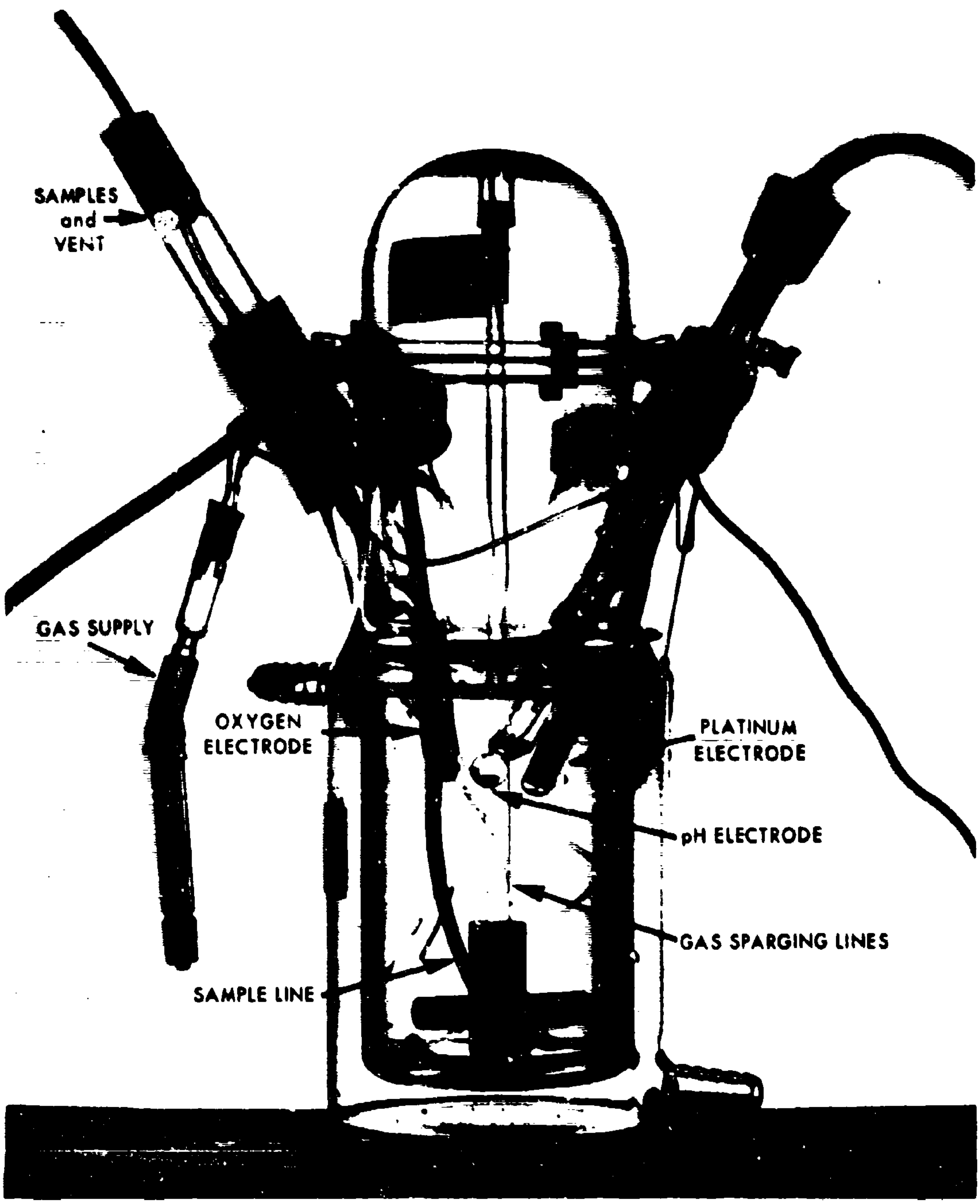


FIGURE 2. Single Instrumented Spinner Flask.

Initial results showed that even with continuous sparging with a gas mixture consisting of 10% oxygen, 5% CO_2, and 85% N_2, the dissolved oxygen tension dropped to near zero when cell populations approached 4×10^6 cells per ml. Because of this problem of maintaining pO_2 values at preselected levels, a control system was designed that maintained pO_2 at desired levels by varying O_2 input to the sparge gas mixture in response to changes in oxygen electrode readings. This system is shown in Figure 3.

Sequential measurements of oxygen tension in each flask could be recorded with stepping switches. Similarly, the redox potential and pH values of each culture could be determined and recorded as shown in Figure 4. The complete controlled environment culture system is illustrated in Figure 5.

The growth curve of L cells obtained in this system with the pO_2 maintained at 9%, together with continuously recorded pH and redox values, is shown in Figure 6. As shown here, relatively uniform values of medium pH and of redox potential were maintained during the growth cycle under controlled pO_2 condition. In our culture system, pH values generally remained within 6.9±0.2 pH units at varied pO_2 levels without further adjustment as long as the CO_2 content of the sparging gas mixture was maintained at 5%. On the other hand, redox potentials clearly reflected differences in pO_2 values. When growth of L cell cultures was compared over a pO_2 range of 2 to 20%, optimum growth was obtained at a pO_2 value of approximately 9% (Fig. 7). This value agrees with results reported by other workers cited earlier. Redox potentials of cultures at pO_2 values ranging from 2 to 20% showed readings, based on a saturated calomel reference electrode, ranging from -25 to +150 millivolts (Fig. 8). It appears, therefore, that the redox potential for optimal growth of L cells is approximately +90 millivolts. This result is in essential agreement with the results obtained by Daniels[8] and Wiles.[5]

Continuing investigation with this equipment will include studies on the effects of varied CO_2 partial pressures as well as effects of varied pH on cell growth and physiology. The equipment is versatile and has many other applications to studies of effects of environment on cell physiology.

In summary, we have described equipment designed to permit study of the effects of environmental variables on the physiology of cultured mammalian cells. In a totally synthetic culture medium and an automatically controlled system, an oxygen partial pressure of approximately 9% was optimal for growth of L cells. The applicability of this system to studies on effects of such variables as pH, pCO_2, and redox potential is evident.

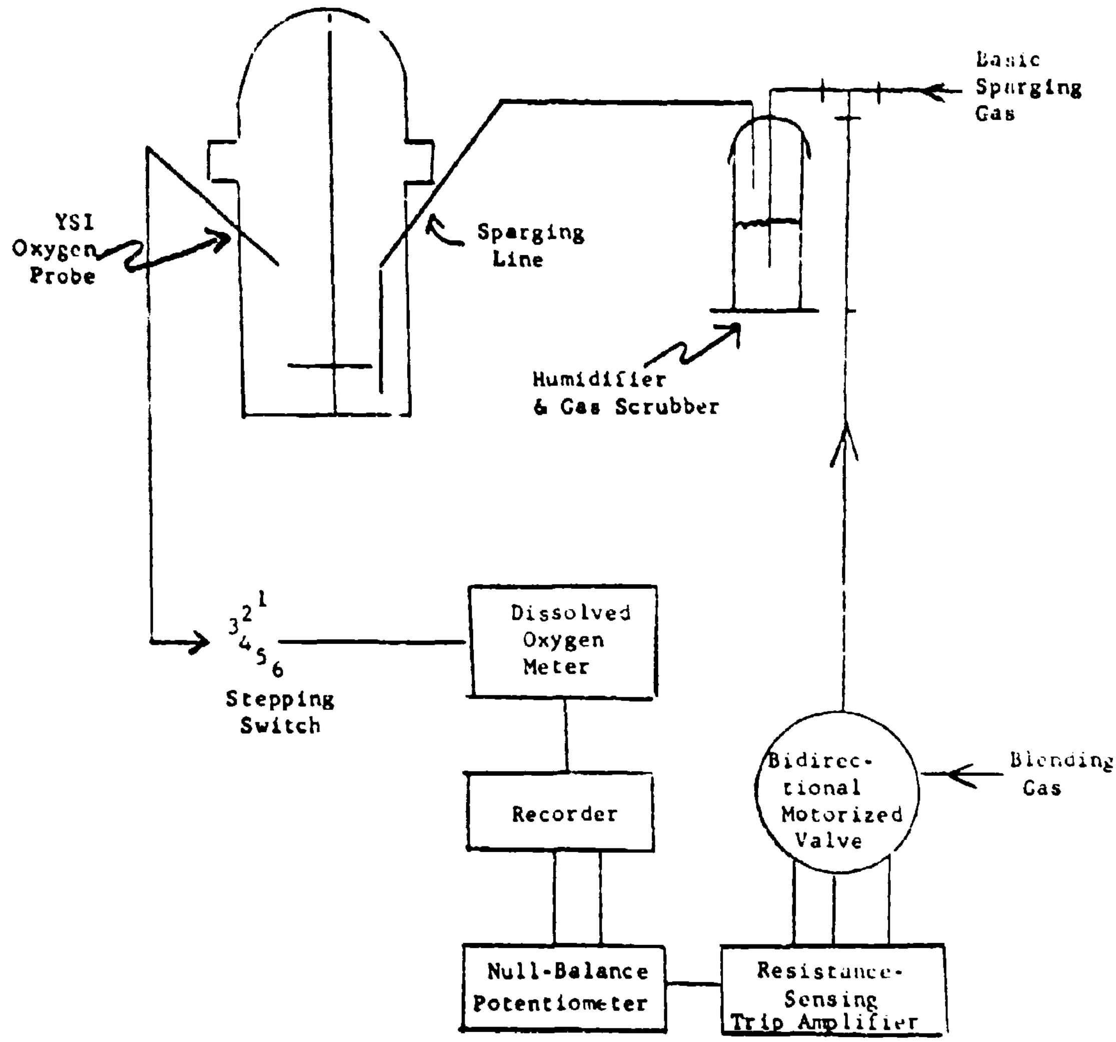


FIGURE 3. Block Diagram of Automated Oxygen Control System for Cell Cultures.

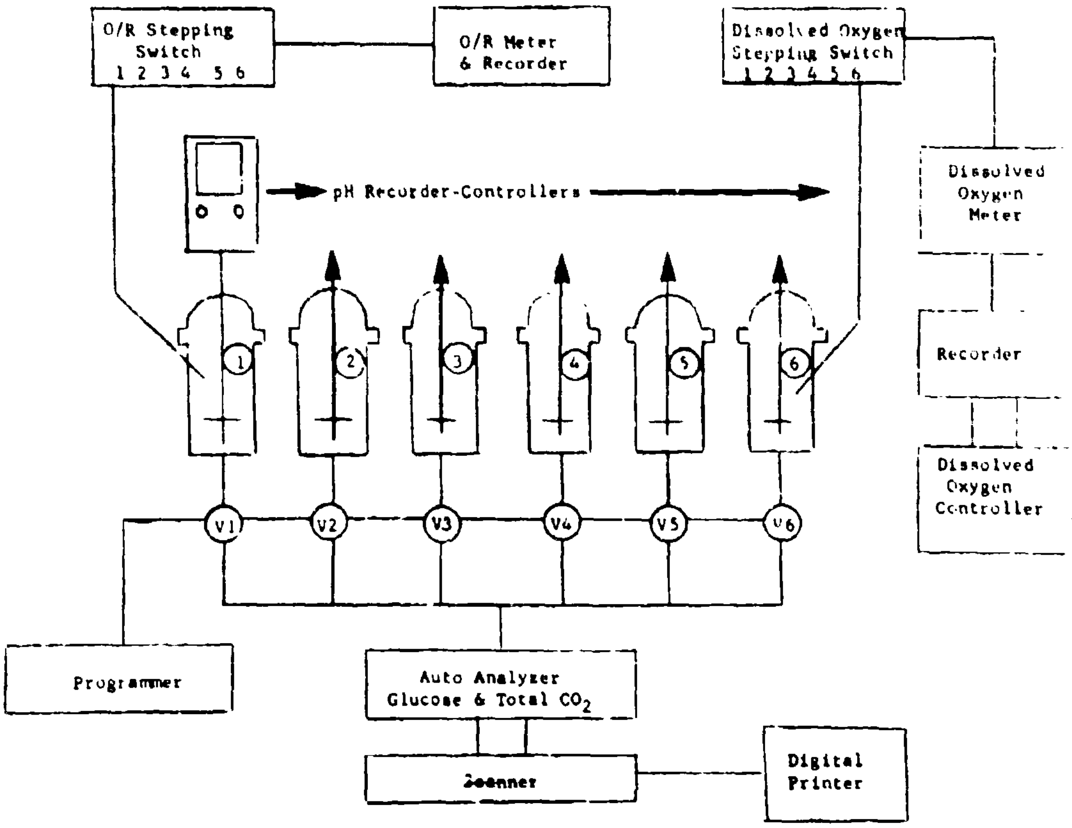


FIGURE 4. Block Diagram of Automated Cell Culture Environmental Control and Monitoring System.

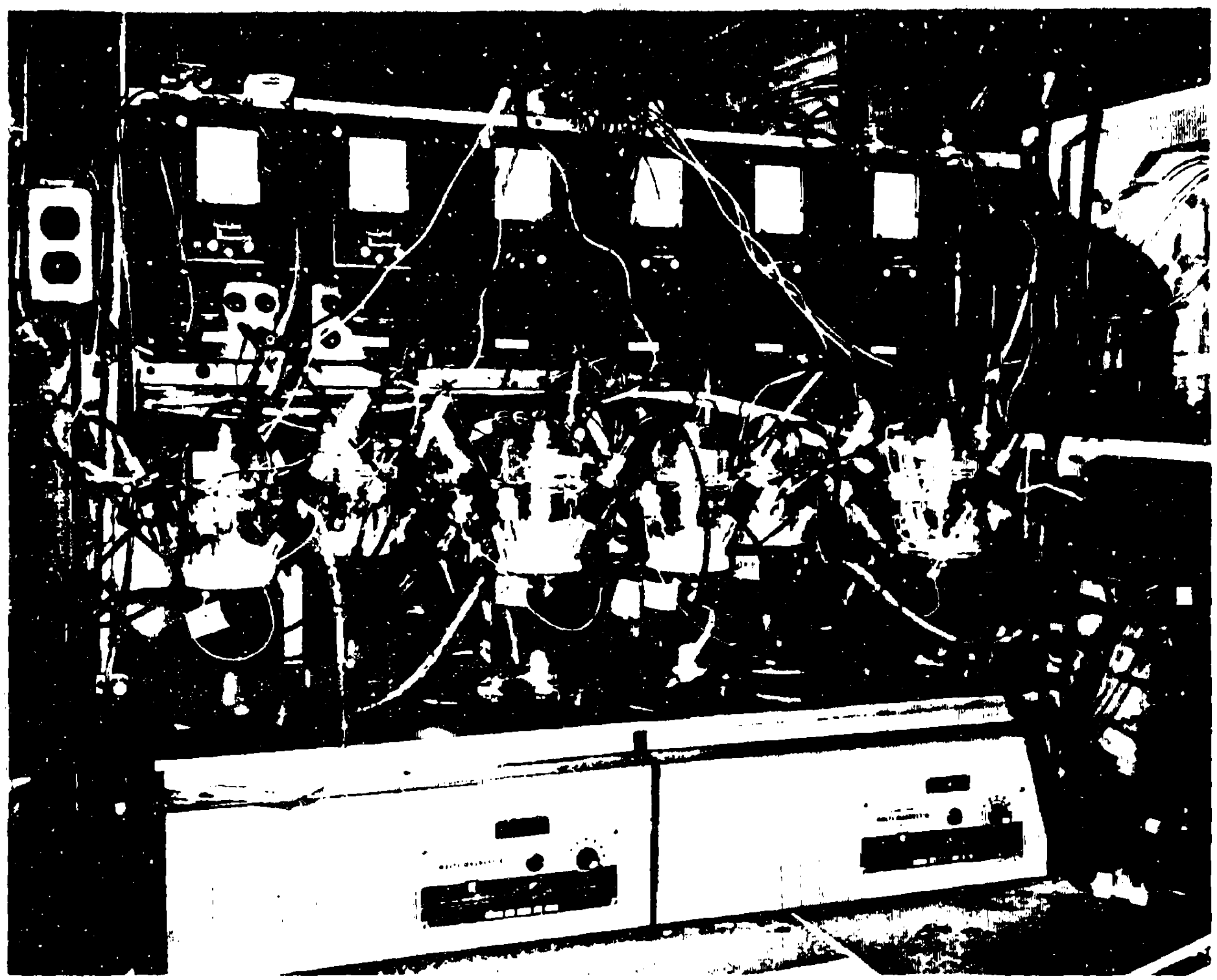


FIGURE 5. Complete Automated Cell Culture Environmental Control and Monitoring System.

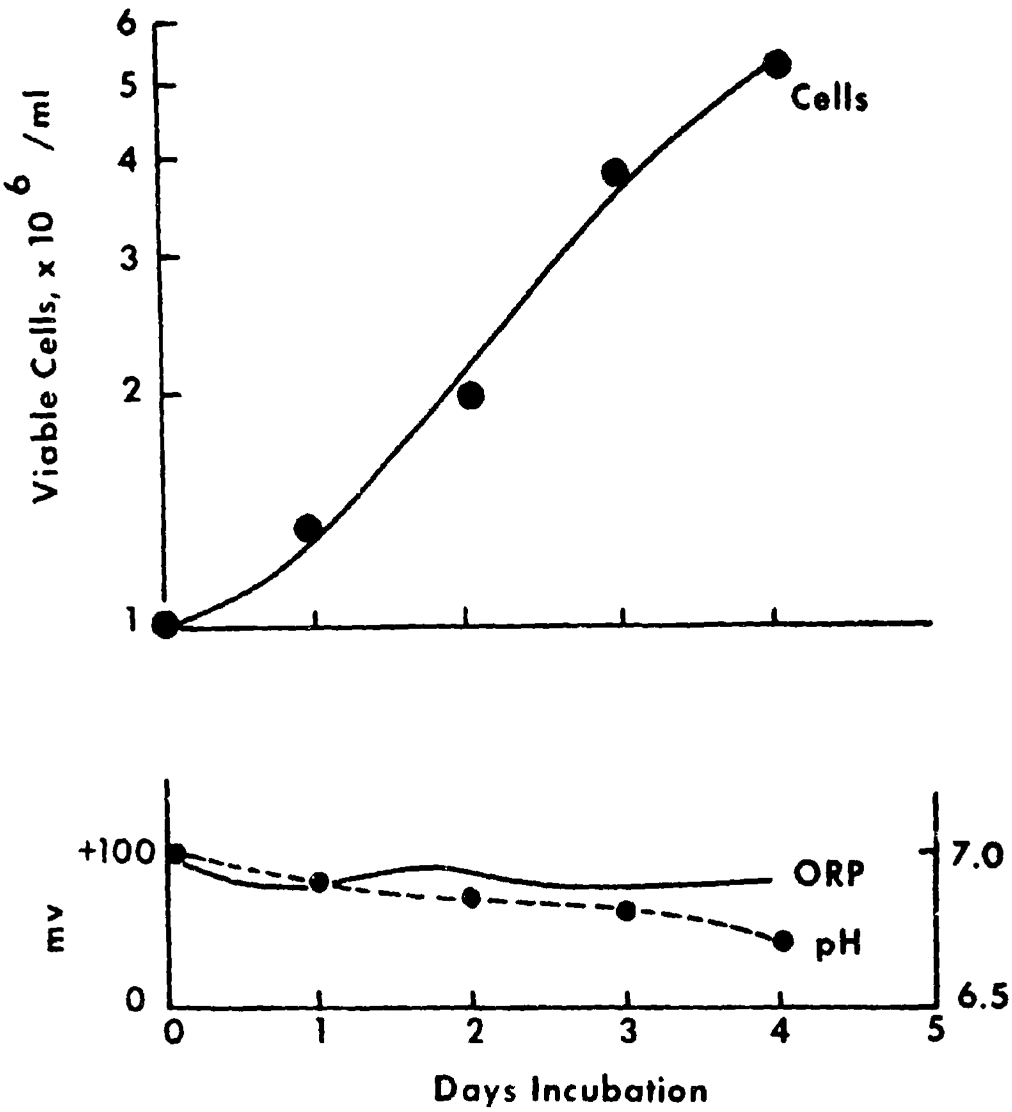


FIGURE 6. Growth, ORP, and pH Values of L Cell Cultures Controlled at 9% Dissolved Oxygen Tension.

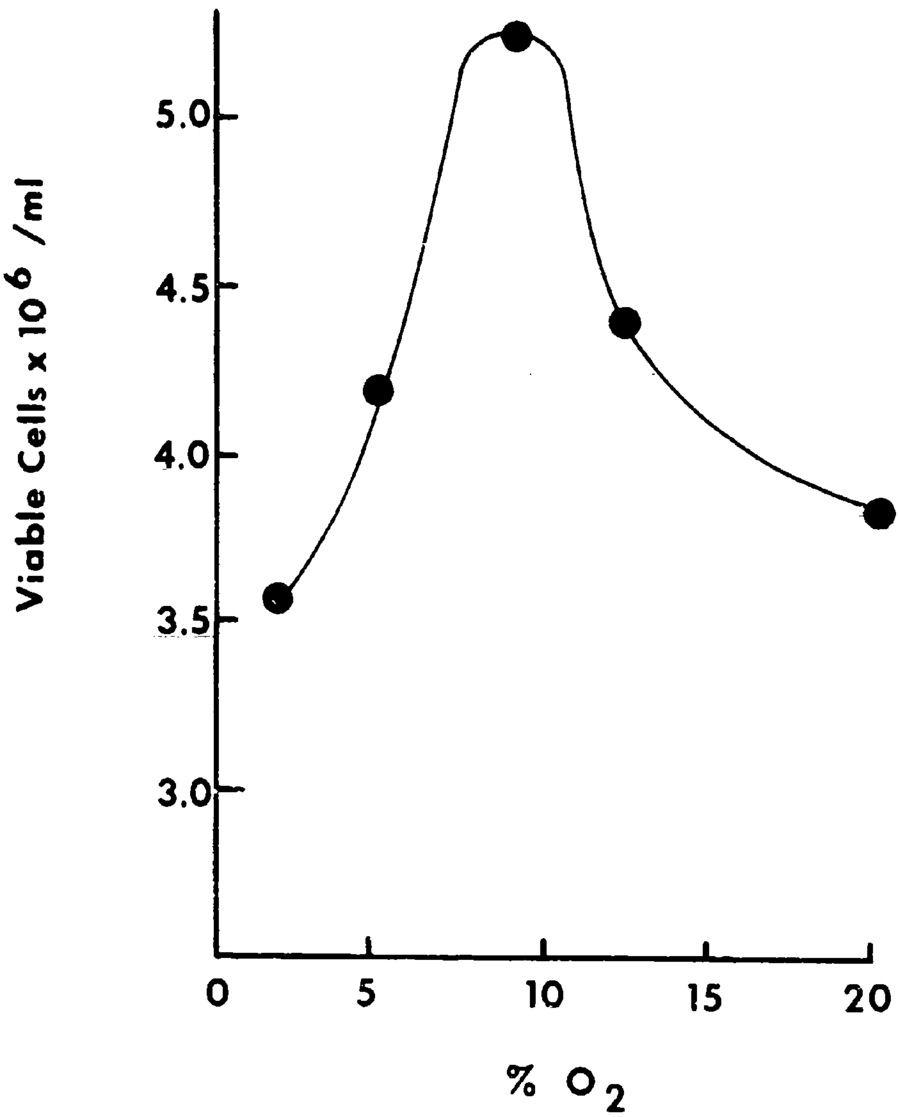


FIGURE 7. Peak Yields of L Cell Cultures Grown At Various Controlled Dissolved Oxygen Tensions.

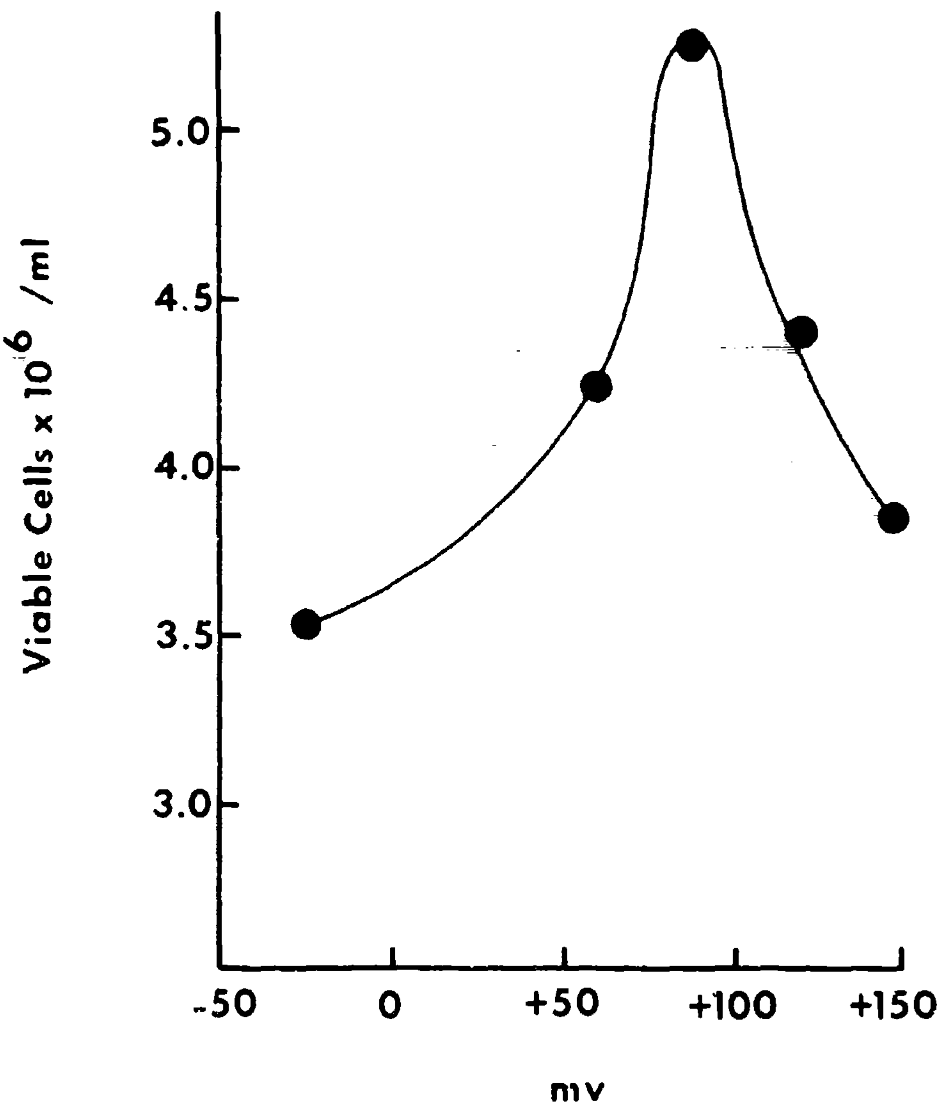


FIGURE 8. Correlation Between Peak Yields and Recorded Redox Potentials of L Cell Cultures.

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L cells						
Chemically defined medium						
Environmental control						
Controlled environment						
Suspension cultures						
Redox and pO_2 relationship						
ORP and pO_2 relationship						
Oxygen tension						

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